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Pre-emergence Control of Six Invasive Winter Annual Grasses with Imazapic and Indaziflam

Derek J. Sebastian, Scott J. Nissen, and Juliana De Souza Rodrigues*

Managing invasive winter annual grasses on noncrop and rangeland remains a constant challenge throughout many regions of the United States. Currently, there are limited management options for controlling winter annual grasses that work consistently, provide multiple years of control, and do not injure desirable plant communities. Imazapic has been one of the most widely used herbicides for downy brome control on rangeland; however, control with imazapic has been inconsistent beyond the application year and perennial grass injury is not uncommon. Indaziflam, a new herbicide mode of action for rangeland weed management, has shown promise in providing long-term downy brome control. A greenhouse study was conducted to compare pre-emergence activity of imazapic and indaziflam on six invasive winter annual grasses: downy brome, cereal or feral rye, jointed goatgrass, Japanese brome, medusahead, and ventenata. For both herbicides, seven rates were used to develop dose-response curves for each species. Log-logistic regression was conducted to determine the herbicide dose required to reduce biomass by 50% (GR_{50} values). Indaziflam was significantly more active across all species compared to imazapic, with the exception of jointed goatgrass. Comparing all species, the GR_{50} values for imazapic were on average 12 times higher than indaziflam. Japanese brome was the most sensitive to both herbicides, whereas jointed goatgrass and feral rye were the most difficult winter annual grasses to control with indaziflam and imazapic, respectively. This research provides evidence of a potential new mode of action for land managers to control the major invasive winter annual grasses.

Nomenclature: Imazapic; indaziflam; cereal or feral rye, *Secale cereale* L.; downy brome, *Bromus tectorum* L.; Japanese brome, *Bromus japonicus* Thunb. ex Murr.; jointed goatgrass, *Aegilops cylindrica* Host.; medusahead, *Taeniatherum caput-medusae* (L.) Nevski; ventenata, *Ventenata dubia* (Leers) Coss. in Dur.

Key words: Dose-response, Great Basin, invasive weed, invasive winter annual grass, rangeland, restoration.

Invasive winter annual grasses are a serious concern in the western United States and continue to spread rapidly across noncrop and rangeland areas displacing native vegetation. Great Basin sagebrush ecosystems that were once primarily perennial plant dominated are being transformed to annual grass-dominated plant communities (Chambers et al. 2014). Exotic winter annual grasses are highly competitive with native perennial grasses and greatly reduce above- and belowground biomass, deplete soil moisture, and reduce native plant diversity (Crawford et al. 2004; D'Antonio and Vitousek 1992; DiTomaso 2000; Haferkamp et al. 1997, 2001; Monaco et al. 2005; Wallace et al. 2015). This can drastically influence the structure and function of these ecosystems (Knapp 1996; Young 1992), while at the same time decrease their resistance and resilience to invasion (Chambers et al. 2014).

As invasive annual grasses continue to increase, effective management becomes critical for restoring and maintaining native rangeland ecosystems. This is particularly true for the over 23 million ha (57 million ac) of public land in the Great Basin and western United States currently infested by annual grasses such as downy brome (*Bromus tectorum* L.) and medusahead [*Taeniatherum caput-medusae* (L.) Nevski] (Duncan et al. 2004; Pellant and Hall 1994). Although downy brome is the most widespread invasive plant in the United States (Duncan et al. 2004), medusahead is the most problematic invasive annual grass found on California rangelands and has been found as far east as Nevada and Utah (Kyser et al. 2012; Monaco et al. 2005; Nafus and

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Management Implications

Invasive winter annual grasses pose a major threat to native plant communities in the United States. The life cycle of these species increases their invasiveness because few native species behave as winter annuals, providing a niche for invasive annual grasses to exploit moisture and nutrients when most desirable perennial plants are dormant. Although downy brome alone infests over 22 million ha of U.S. rangeland, there are five other invasive winter annual grasses that cause significant economic and ecological impacts: feral rye, Japanese brome, jointed goatgrass, medusahead, and ventenata.

Currently, acetolactate synthase- (ALS) inhibiting herbicides such as imazapic and rimsulfuron are used for selective winter annual grass control, whereas nonselective herbicides like glyphosate are also recommended for dormant season applications (late fall or early spring). Unfortunately, none of these herbicides provide consistent control beyond 1 yr after treatment (YAT), resulting in rapid reinvasion of treated areas via the soil seed bank. Indaziflam (Bayer CropScience), a cellulose biosynthesis-inhibiting herbicide, is a new mode of action for invasive winter annual grass management. Previous field research demonstrated that indaziflam provided excellent downy brome and feral rye control 2 and 3 yr after treatment compared to imazapic. Two applications of indaziflam over a 5-yr period could substantially reduce or possibly eliminate the winter annual grass seed from the soil seed bank. The objective of this study was to evaluate indaziflam's potential to control other problematic invasive winter annual grasses found in the United States and compare its activity to the most commonly used herbicide, imazapic. The herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) was calculated for each invasive winter annual grass. In the greenhouse, indaziflam was significantly more active against all winter annual grasses compared to imazapic, with jointed goatgrass as an exception. Averaged across all invasive winter annual grasses, imazapic GR_{50} values were 12 times greater compared to indaziflam.

The potential for long-term downy brome management is very encouraging; however, downy brome is only one species in a suite of winter annual grasses that threaten native ecosystems from the Great Plains to the Pacific Coast. This research indicates that indaziflam is active in controlling a range of winter annual grasses, and based on what we know about the soil seed bank of these species, indaziflam could be a key component in providing long-term management. Our findings provide evidence that indaziflam could be an alternative strategy for controlling invasive winter annual grasses, including relatively new invaders such as medusahead and ventenata. Additional field research is needed to determine if indaziflam provides the long-term control of ventenata, medusahead, jointed goatgrass, and Japanese brome that has been previously reported with downy brome and feral rye.

Davies 2014) (Figure 1). Other invasive annual grasses that represent substantial threats to natural ecosystems include: cereal or feral rye (*Secale cereale* L.) (Ellstrand et al. 2010; Western Coordinating Committee 077 2005; White et al. 2006), jointed goatgrass (*Aegilops cylindrica* Host.) (Beck et al. 1995; National Jointed Goatgrass Research Program 2009), Japanese or field brome (*Bromus japonicus* Thunb. or *Bromus arvensis* L.) (Beck 2009; Haferkamp et al. 2001), and ventenata [*Ventenata dubia* (Leers) Coss. in Dur.] (Bansal et al. 2014; Northam and Callihan 1994; Wallace et al. 2015; Wolff 2013) (Figure 1).

Japanese brome is widespread throughout the United States, but is more prolific in the western United States and northern Great Plains (Haferkamp et al. 2001). Feral rye and jointed goatgrass are two distinctive invasive winter annual grasses that result in high wheat yield losses and also infest areas surrounding these cropping systems. Populations continue to spread to noncropland areas such as roadsides and overgrazed pastures (Beck et al. 1995; National Jointed Goatgrass Research Program 2009; Western Coordinating Committee 077 2005). Ventenata, commonly referred to as wiregrass or North Africa grass, currently invades areas mainly in the Intermountain Pacific Northwest (Pavek et al. 2011; Wallace et al. 2015; Wolff 2013). Ventenata is an increasing threat to recently disturbed perennial grass systems and has even been shown to displace other invasive annual grasses such as downy brome and medusahead (Wallace et al. 2015). Effective, long-term control strategies are crucial to proactively manage this localized species in order to decrease further spread (Wallace and Prather 2016).

Disturbed soils provide conditions for invasive winter annual grasses to establish and spread efficiently; however, it is common for species such as downy brome and medusahead to spread into nondisturbed rangeland via seed dispersal mechanisms (Davies et al. 2013; Morrow and Stahlman 1984). Species evaluated in this study rapidly accumulate dense thatch layers that provide microhabitats that help to perpetuate the invasive species (Wallace et al. 2015; Young 1992). Downy brome and medusahead thatch layers are highly susceptible to fires and suppress germination and establishment of native rangeland species (Kyser et al. 2013; Mangold et al. 2013; Nafus and Davies 2014; Young 1992). The accumulation of these fine fuels shortens fire return intervals, resulting in the displacement of sagebrush ecosystems that are habitat to species such as the greater sage grouse (Chambers et al. 2014; Crawford et al. 2004; D'Antonio and Vitousek 1992; Mangold et al. 2013; Whisenant 1990).

Among the currently available management strategies, herbicides are the most common method used to control invasive winter annual grasses (DiTomaso 2000). Three commonly recommended herbicide treatments and application rates for invasive winter annual grass control in the United States include imazapic (Plateau, BASF, 105 g ai ha^{-1} (1.5 oz ai ac^{-1}) with 201 g ai ha^{-1} annual maximum) (Kessler et al. 2015; Monaco et al. 2005; Sebastian et al. 2016b; Wallace and Prather 2016), rimsulfuron (Matrix, Bayer CropScience, 53 g ai ha^{-1}) (Sebastian et al. 2016b; Wallace and Prather 2016), and glyphosate (Roundup Weathermax, Monsanto, 420 g ae ha^{-1}) (Kyser et al.

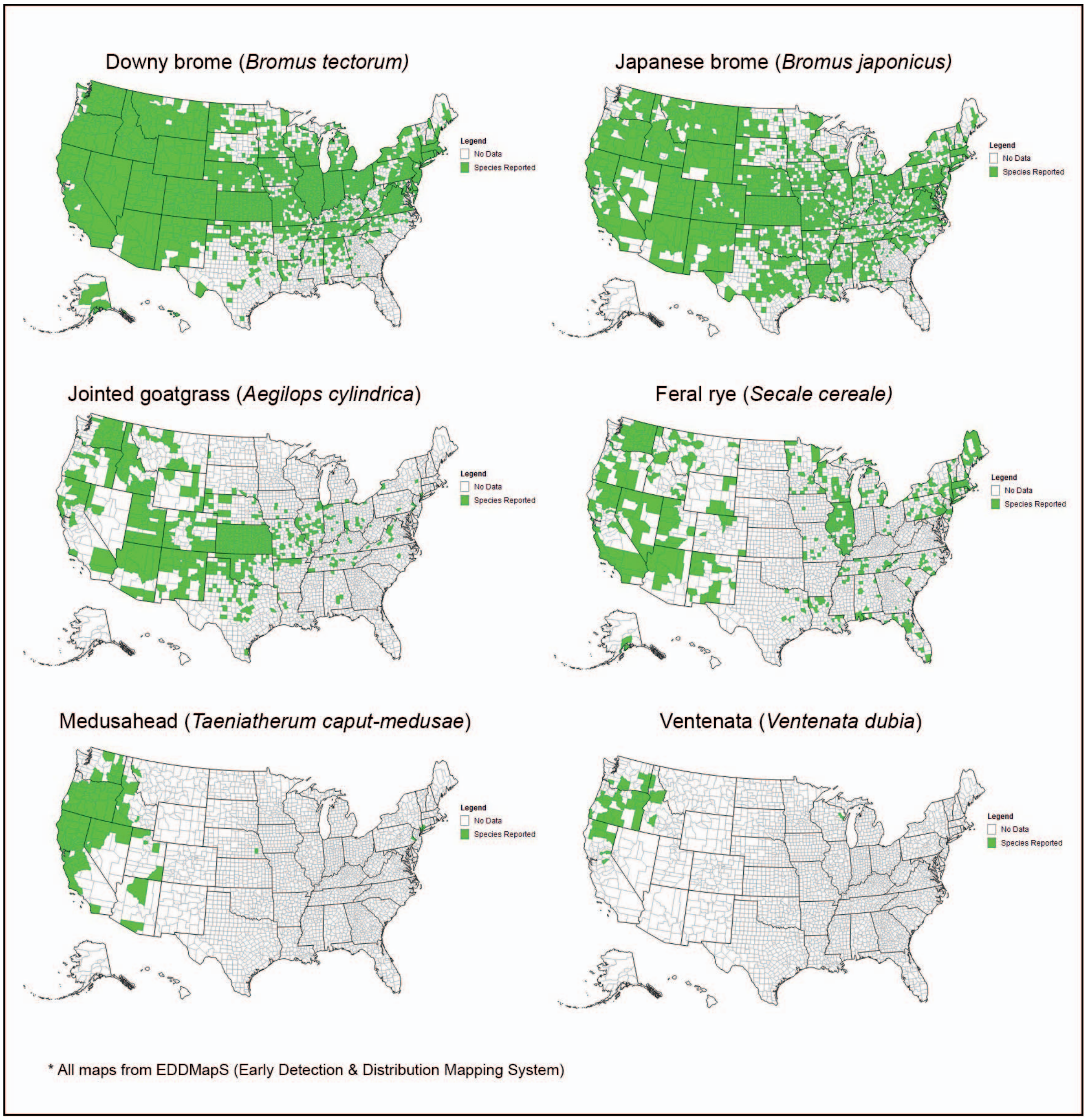

Figure 1. U.S. distribution of the six invasive winter annual grasses evaluated in this study. Maps were taken from the EDDMapS (Early Detection and Distribution Mapping System, https://www.eddmaps.org/distribution/). (Color for this figure is available in the online version of this article.)

2013). Imazapic and rimsulfuron provided limited residual control and lack consistency beyond the initial application year (Hirsch et al. 2012; Kyser et al. 2007, 2012, 2013; Mangold et al. 2013; Monaco et al. 2005; Morris et al. 2009). These herbicides, including glyphosate, can also injure co-occurring species, depending on application timing (Baker et al. 2009; Davies and Sheley 2011; Shinn and Thill 2004; Wallace and Prather 2016). Efforts to restore native plant communities impacted by invasive winter annual grasses are frequently unsuccessful due to rapid reinvasion from the soil seed bank (Davies and Johnson 2011); therefore, new management strategies that address the soil seed bank are needed.

Indaziflam (Esplanade, Bayer CropScience), a new preemergence herbicide registered in the United States for the control of annual grass and broadleaf weeds in citrus, grape, and tree nut crops, could provide the residual weed control necessary to limit reinvasion. This herbicide belongs to the alkylazine class and is the first cellulose-biosynthesis inhibitor (CBI) that could potentially be used for controlling invasive winter annual grasses found on noncropland in the United States. Bayer CropScience has developed a supplemental label for the release or restoration of desirable vegetation on noncrop areas such as parks and open space, wildlife management areas, fire rehabilitation areas, and other nongrazed sites (May 2016). Studies are currently being conducted to support a grazing tolerance; therefore, current indaziflam treatments are limited to sites not grazed by domestic livestock. Indaziflam has a relatively long half-life (> 150 d) in the soil. Application rates of indaziflam range between 51 and 102 g ai ha^{-1} with a yearly maximum of 146 g ai ha^{-1} (Brabham et al. 2014; Tompkins 2010), whereas the recommended rates for residual winter annual grass control are 73 and 102 g ai ha^{-1}. In field experiments conducted in Colorado, established native perennial grasses, forbs, and shrubs were tolerant to indaziflam (Sebastian and Nissen 2016). Field studies have shown that indaziflam provides superior downy brome and feral rye control compared to imazapic (Sebastian et al. 2014, 2016b). Imazapic and indaziflam applied PRE provided similar downy brome control 1 yr after treatment (YAT); however, indaziflam provided 83 to 100% downy brome control 2 and 3 YAT (Sebastian et al. 2016b). This level of residual control could help to manage the soil seed bank of invasive winter annual grasses, thus limiting reinvasion. There is currently no published literature evaluating indaziflam's activity on invasive winter annual grasses other than downy brome.

The main objective of this research was to compare imazapic and indaziflam activity on invasive winter annual grasses found in the western United States using greenhouse dose-response experiments. We hypothesized that indaziflam could provide increased winter annual grass control across all species compared to imazapic. These greenhouse experiments represent the most comprehensive analysis comparing the currently recommended herbicide, imazapic, with indaziflam.

Materials and Methods

Study Species. A greenhouse dose-response was conducted to compare the sensitivity of six invasive winter annual grasses to imazapic and indaziflam (Figure 1). All species were collected from their invaded range: downy brome and feral rye (Larimer County, CO), Japanese brome (Jefferson County, CO), jointed goatgrass (Phillips County, Colorado), medusahead (Yuba County, California), and ventenata (Latah County, Idaho). Seeds were collected from senesced plants the year prior to this study and stored at –4 C (25 F) until planting in 2015.

Seeds were planted in plastic containers (17 cm by 12 cm by 6 cm [7 in by 5 in by 2 in) filled with field soil. The field soil was an Otero sandy clay loam (Coarse-loamy, mixed [calcareous], mesic Aridic Ustorthents) with 3.9% OM and pH 7.7. Seeding densities were adjusted based on germinability to reach a target density of 40 plants pot^{-1}. All species were planted at a depth of 0.5 cm.

Experimental Design. The experimental design was a factorial with six herbicide rates and a nontreated arranged in a completely randomized design with three replicates. The study was repeated July 27, 2015 and September 29, 2015. A preliminary study was conducted to approximate a range of doses that would best fit a logistic regression model for each herbicide and species. It is not unusual for both pre-emergence and postemergence herbicides to be more active (provide control at lower than labeled rates) in the greenhouse with ideal environmental conditions, so it was not surprising to us that herbicide doses for the regression analysis were much lower than recommended field use rates. Imazapic was applied at rates of 0, 2.2, 4.4, 8.8, 17.5, 35.0, and 70.1 g ai ha^{-1} for downy brome, Japanese brome, medusahead, and ventenata; whereas for feral rye rates were 0, 8.8, 13.1, 17.5, 35.0, 70.1, and 140.2 g ai ha^{-1} and for jointed goatgrass rates were 0, 4.4, 8.8, 17.5, 35.0, 70.1, 140.2, and 280.4 g ai ha^{-1}. Indaziflam was applied at rates of 0, 0.2, 0.4, 0.7, 1.5, 2.9, and 5.9 g ai ha^{-1} for all species except jointed goatgrass where rates of 0, 0.7, 1.5, 2.9, 5.9, 11.7, and 23.4 g ai ha^{-1} were used. Herbicides were applied using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray nozzle (TeeJet Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L ha^{-1} (20 gal ac^{-1}) at 172 kPa (25 lb in^{-2}).

Following herbicide treatments, plants were maintained in a greenhouse with a 25/20 C day/night temperature regime at an approximate 60% relative humidity. Natural light was supplemented with high-intensity discharge lamps to give a 15-h photoperiod. Plants were subirrigated weekly and misted daily to reduce soil crusting. Aboveground plant biomass was harvested at the soil surface 4 wk after treatment (WAT) and dried for 5 d at 60 C before recording dry weights.

Data Analysis. Total dry weights for each treatment were converted to a percentage of the biomass in the nontreated. Data were first analyzed using the PROC MIXED method in SAS 9.3 with treatment as a fixed effect and experiment and replicate as random effects (SAS Institute 2010). After failing to reject the null hypothesis of equal variance, the repeated studies were combined for analysis. Graphpad Prism 6 was used to determine imazapic and indaziflam rates required to reduce plant dry biomass by 50% (GR_{50}) for each invasive winter annual grass. The four-parameter log-logistic regression equation regressing biomass as a percent of the nontreated with herbicide concentration is given in Equation 1:

$$Y = C + \frac{(D - C)}{1 + 10^{(Log\mathrm{GR}_{50} - X)\cdot b}} \qquad [1]$$

where C is the lower limit of response, D is the upper limit

Table 1. Imazapic and indaziflam rates resulting in 50% reduction (GR_{50}) in growth of six invasive winter annual grasses. Values were calculated using log-logistic regression. ($GR_{50} \pm$ SE).

Invasive winter annual grass	Imazapic GR_{50}	Indaziflam GR_{50}	Imazapic/ Indaziflam GR_{50} Ratio	P value[a]
	g ai ha^{-1}	g ai ha^{-1}		
Downy brome	2.71 ± 0.10	0.23 ± 0.07	11.78	< 0.0001*
Feral rye	24.37 ± 0.07	0.56 ± 0.06	43.52	< 0.0001*
Japanese brome	1.86 ± 0.08	0.19 ± 0.05	9.80	0.0004*
Jointed goatgrass	13.96 ± 4.70	7.37 ± 3.58	1.89	0.6447
Medusahead	2.07 ± 0.12	0.36 ± 0.09	5.75	< 0.0001*
Ventenata	7.08 ± 0.13	$0.44 + 0.09$	16.10	< 0.0001*

[a] Within each row, P values comparing imazapic and indaziflam GR_{50} values (*significance according to Fisher's Protected LSD at the 5% level of probability).

of response, *b* the slope, and GR_{50} is the herbicide rate resulting in 50% reduction in biomass. Means were separated for each invasive winter annual grass to determine significant differences in GR_{50} values, using Fisher's Protected LSD test at the 5% level of probability. The recommended use rates for indaziflam range from 70 to 97% (73 and 102 g ai ha^{-1}) of the commonly recommended imazapic use rate (105 g ai ha^{-1}); therefore, pre-emergence control was compared directly using GR_{50} estimates.

Results and Discussion

Indaziflam was significantly more active against all winter annual grasses compared to imazapic (Figure 2), with the exception of jointed goatgrass. Although indaziflam's GR_{50} value for jointed goatgrass was approximately half that of imazapic, this was the only species in which the GR_{50} values were not significantly different ($P = 0.6447$) (Table 1). We used these data to confirm results from previous field experiments comparing these two herbicides (Sebastian and Nissen 2016; Sebastian et al. 2016b) and make inferences about how these data can be applied to other invasive winter annual grasses that have not been evaluated under field conditions (Table 1).

The downy brome GR_{50} values were significantly higher for imazapic (2.71 ± 0.10 g ai ha^{-1}) as compared to indaziflam (0.23 ± 0.07 g ai ha^{-1}) (Figure 2). Furthermore, Japanese brome showed the greatest sensitivity ($GR_{50} = 0.19$ g ai ha^{-1}) to indaziflam, whereas jointed goatgrass ($GR_{50} = 7.37$ g ai ha^{-1}) was the least sensitive (Table 1). For imazapic, Japanese brome showed the greatest sensitivity ($GR_{50} = 1.86$ g ai ha^{-1}), and feral rye ($GR_{50} = 24.37$ g ai ha^{-1}) was the least sensitive (Table 1). The indaziflam GR_{50} values for medusahead and ventenata were 6 and 16 times lower compared to imazapic, respectively ($P < 0.0001$, Figure 2).

Ventenata and medusahead are relatively new invaders to the western United States (Wallace et al. 2015), increasing the importance of reducing further spread of these species to highly susceptible areas such as the Great Basin. In these areas, productive wildlife habitat, including intact sagebrush landscapes, are crucial for species such as the sage grouse (*Centrocercus urophasianus* and *C. minimus*) (Chambers et al. 2014; Crawford et al. 2004). Ventenata in particular poses a major threat to the native grassland ecosystems of the Palouse Prairie of eastern Washington and northern Idaho (Wallace et al. 2015). Indaziflam appears to be an alternative control option for managing these two invasive annual grasses.

Indaziflam's significantly lower GR_{50} values compared to imazapic provides some evidence to support the idea that several years of residual control could be possible with indaziflam for these other winter annual grasses in a manner similar to what has been reported for downy brome (Sebastian et al. 2016b). Previous studies have shown differences in relative potency when comparing indaziflam and flumioxazin for kochia [*Kochia scoparia* (L.) Schrad.] control; differences were attributed to variances in herbicide absorption and mode of action (Sebastian et al. 2016a). Indaziflam controls weeds as the primary root emerges from the seed, whereas ALS inhibitors must be absorbed by plant roots, translocated to meristematic regions, and then inhibit fatty acid production in the chloroplast.

Some of the tested winter annual grasses have shown differential responses to other herbicides. Downy brome, feral rye, and jointed goatgrass responded differently to imazamox (Pester et al. 2000). The differential response of these species to imazamox was a result of differences in translocation, metabolism, or absorption. Jointed goatgrass was found to be the most susceptible to imazamox, downy brome control was intermediate, and feral rye was the most tolerant (Pester et al. 2001). Similarly, differences in herbicide absorption and mode of action between imazapic

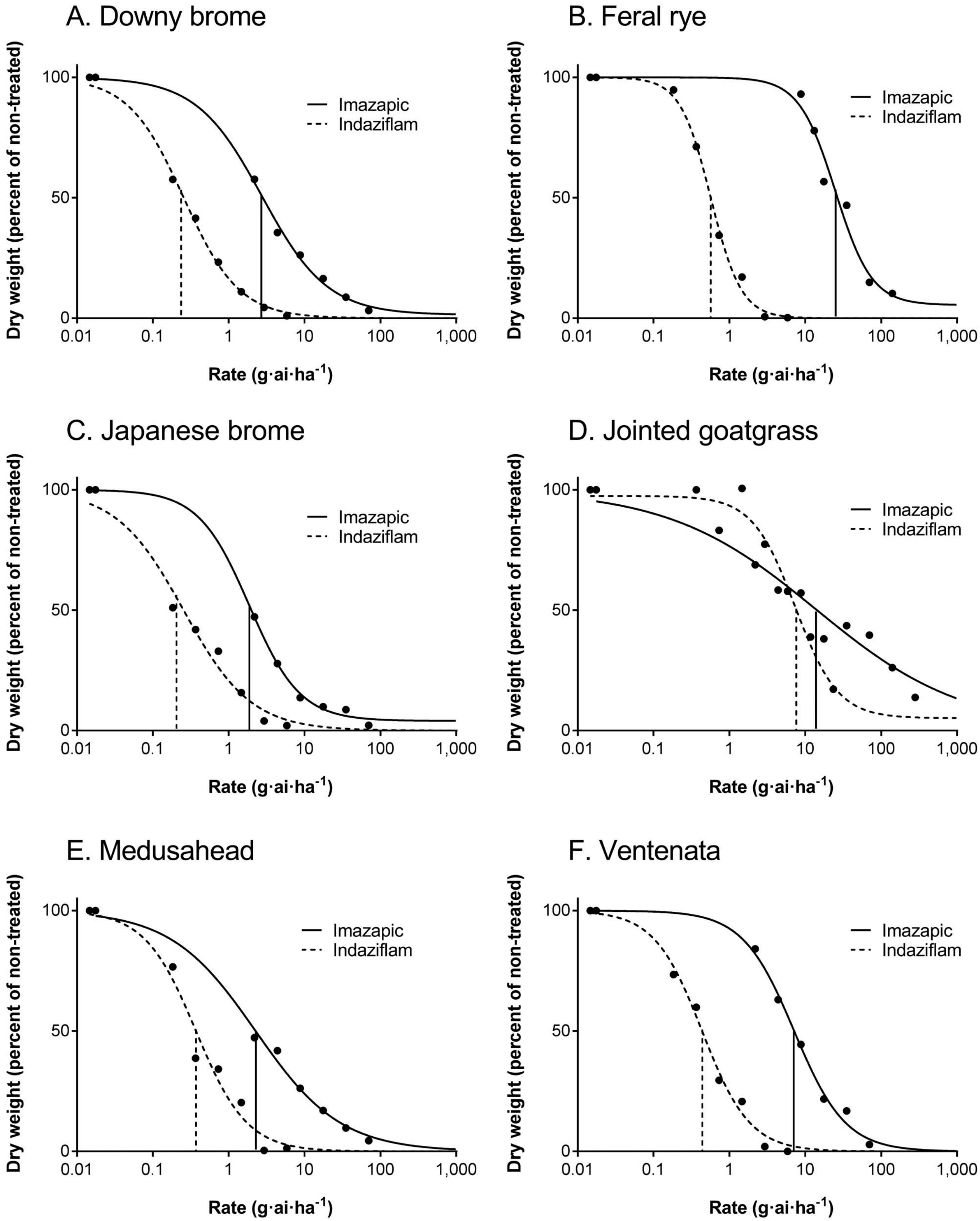

Figure 2. Response of (A) downy brome, (B) feral rye, (C) Japanese brome, (D) jointed goatgrass, (E) medusahead, and (F) ventenata to imazapic and indaziflam. Dose response curves were fit using four parameter log-logistic regression. Mean values of six replications are plotted. Vertical lines represent the herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) for each species and herbicide.

and indaziflam could be responsible for the difference in relative potency. Other contributing factors could be the herbicides' water solubility and degradation by soil microbes (longer half-life in the soil). Indaziflam has a longer average soil half-life ($>$ 150 d) and lower water solubility (4.4 mg L^{-1} [5.5×10^{-4} oz gal^{-1}] at pH = 4 and 2.8 mg L^{-1} at pH = 9) than imazapic (120 d, 2,200 mg L^{-1}). These characteristics in combination with different modes of action could be the major contributing factors resulting in indaziflam's long-term residual winter annual grass control and increased phytotoxicity compared to imazapic (WSSA 2014).

It is well documented that invasive winter annual grasses continue to invade sagebrush and grassland ecosystems in the United States, resulting in the displacement of native vegetation, reduction in quality wildlife habitat (Baker et al. 2009; Chambers et al. 2014; Kyser et al. 2013), decreased fire-return intervals (Balch et al. 2013; Billings 1994; Chambers et al. 2014; D'Antonio and Vitousek 1992), and altered resistance and resilience of these native ecosystems (Chambers et al. 2014). Due to the magnitude of invasive winter annual grass infestations and the potential for further spread, new herbicidal modes of action should be considered. Indaziflam showed increased phytotoxicity compared to imazapic across all six species (Table 1; Figure 2). These data suggest that indaziflam is more biologically active than imazapic on these species and supports results from field studies (Sebastian et al. 2016b).

It is possible that plants evaluated in the greenhouse are more susceptible to herbicide injury; therefore, further research is necessary to determine if these findings are reproducible under field conditions. Imazapic and indaziflam bioavailability have been shown to be affected by differences in soil properties and soil moisture (Adolfo et al. 2005; Alonso et al. 2011; Inoue et al. 2009; Sebastian et al. 2016a), so field studies should be conducted across the western United States.

Additional studies should also evaluate indaziflam's impacts on annual grassland systems in regions such as California. Over the last few centuries, native perennial vegetation has significantly declined due to invasive species such as downy brome, medusahead, and yellow starthistle (*Centaurea solstitialis* L.) (DiTomaso et al. 2007). In California's coastal ranges, central valley, and Sierra Nevada foothills, over 73% of the major invasive nonnative species are winter annuals (DiTomaso et al. 2007). The current study showed that indaziflam controls a wide range of winter annual grasses; therefore, studies should be conducted to evaluate the potential utility of indaziflam to convert these sites to native perennial bunchgrasses (Enloe et al. 2005; Morghan et al. 2005).

The information presented in this study will be beneficial to land managers throughout the western United States who are seeking new herbicides to control invasive winter annual grasses. These data suggest that indaziflam provides increased winter annual grass control at field application rates comparable to imazapic, and might provide residual control similar to previous studies conducted on downy brome (Sebastian and Nissen 2016; Sebastian et al. 2016b). Additional field-scale research is necessary to evaluate indaziflam's potential for long-term control of other invasive winter annual grass. Areas infested by these invasive grasses are large and are continuing to spread (Figure 1). Land managers remain in need of better tools that can control multiple species, while still having the option to re-establish or protect native plant communities. This study provides the first evidence that indaziflam could control a suite of invasive winter annual grasses.

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